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# Art. V.—*Mechanism of Abnormal and Pathological Growth: A Review.*

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## Abstract.

Recent work is reviewed on the mechanism of abnormal and pathological growth in plants with special reference to those effects induced by *Bacterium tumefaciens*, *Bacterium solanacearum* and *Rhizobium* spp. The hypothesis that indole-3-acetic acid, produced by the organism in the plant, induces the cell proliferation or other stimulation effect is regarded as unlikely.

While the phenomena still strongly suggest the working of a growth-substance mechanism, researches so far have not substantiated the alternative hypothesis that the physical presence of the bacteria stimulate the plant to increased production of growth-substance leading to cell proliferation.

Other trends in recent research on this problem are indicated.

In a number of plant infections, growth correlations are disturbed, such disturbance being reflected by overgrowth, inhibition of growth, development of new organs or growth movements. For many years these conditions have been used in symptomatology without more than a passing suggestion as to how they were brought about. With the discovery of the auxins and other synthetic growth-substances and the recognition of the similar effects they are able to produce in healthy plants, attention has been increasingly directed toward the elucidation of the mechanism of abnormal and pathological growths.

The original viewpoint was, that since growth-substances were so important in the normal development of plant tissues, the finding of their presence in unusual amounts in certain abnormal tissues would indicate that they were responsible for these conditions. With increasing research in this field this viewpoint has undergone some modification. The present review represents a summary of progress made and of present trends.

## Types of Abnormal and Pathological Growth.

The literature in plant pathology contains numerous references to abnormal conditions induced in plants as a result of fungal, bacterial and virus infection. These conditions may arise from

successful parasitism or from controlled parasitism and may be delimited as follows:—

*Overgrowth.*

(*a*) Galls, tubercles or callus formation due to rapid multiplication of cells (hyperplasia) and increase in cell size (hypertrophy), whereby a more or less irregular overgrowth appears on shoot or root. Examples include Crown Gall, Club Root, Maize Smut, Rust Galls, Fiji Disease of Sugar Cane, Root Tubercles of Leguminosae and Swollen Leaf Teeth of Ardisia.

(*b*) Stimulation of cambial and other meristematic centres leading to abnormalities in size and number of parts. Examples include Witches Brooms, Fasciation, Crown Gall, Hairy Root, Big Bud of Tomato, Virus enations.

*Adventitious Root Formation.*

Roots arising in unaccustomed places on the stems of intact plants. These occur in Bacterial Wilt of Solanaceae, Crown Gall, Fusarium Wilt of Tomato and Bacterial Canker of Tomato.

*Growth Movements.*

Nastic Responses. Change of position of a bilaterally symmetrical organ due to differential growth. Epinasty and hyponasty, expressing conditions where the upper or lower side respectively of a dorsi-ventral leaf grows faster than the other, leading to a downward or an upward bending of the leaf. Examples include Bacterial Wilt of Solanaceae, Crown Gall, Fusarium Wilt of Tomato and Rose Wilt Virus.

Many of the abnormal growth effects listed above may occur in the same plant as a disease runs its course.

The fundamental problem in all these cases is the explanation of the physiological cause of the atypical cell multiplication and organ formation and movement. This has been pointed out by Riker and Berge (1935), and Riker (1939) in their studies on Crown Gall. Investigation so far has been confined to analysis of relatively few of the atypical growth effects. For the better appreciation of the investigations to be described, it appears desirable to outline briefly the auxin theory of growth, salient features of the effects induced by certain synthetic growth substances and the essential techniques involved.

## Outline of the Auxin Theory of Growth and Effects on Plants of Certain Synthetic Growth-substances.

The idea that a hormone is concerned in growth and growth-movements goes back over a quarter of a century, but success in obtaining an active substance from the coleoptile tip of *Avena* was first obtained by Went (1928). His fundamental experiment was to extract growth-substance by cutting off the tips of

oat coleoptiles and placing them upon a small rectangular plate of moist agar. After one to two hours the tips were removed and the agar plate cut up into blocks of equal size and placed on one side of the stumps of decapitated coleoptiles. The result was a curvature away from the agar blocks (negative curvature), indicating that some chemical growth-promoting substance had diffused out of the tips into the agar and then out of this into the decapitated coleoptiles. The degree of curvature was proportional, within limits, to the concentration of the substance in the agar blocks and the method has been widely applied in the comparison of the amounts of growth-substance in different plant parts and the interpretation of tropic phenomena. There appears little doubt that cell elongation is promoted by the presence of the plant growth-substance. On the other hand the application of this same substance to roots retards their growth.

By another line of research Nielsen (1928, 1930), demonstrated that a fungus, *Rhizopus suinus*, excreted into its culture medium a substance which could be extracted with ether and which induced curvature in *Avena*. A similarly active substance was also obtained from bacteria by Boysen-Jensen (1931). This substance, which was also found in urine (Kogl, Haagen-Smit and Erxleben, 1934), was shown by Kogl and Kostermans (1934), and Thimann (1935) to be identical with indole-3-acetic acid. The name hetero-auxin was coined for it. Prior to the identification of hetero-auxin, Kogl and Haagen-Smit (1931), found human urine to be rich in an active substance, since isolated in crystalline form and named auxin. Later Kogl, Erxleben and Haagen-Smit (1934), discovered and isolated a second active substance (distinct also from hetero-auxin) in urine. The original auxin was then designated auxin "a" ($C_{18}H_{32}O_5$) and the new one auxin "b" ($C_{18}H_{30}O_4$). These with hetero-auxin ($C_{10}H_9O_2N$) comprise the auxin group. All three are active in the *Avena* test. Hetero-auxin can be readily synthesized, but so far the other two have not.

Subsequently a variety of growth-substances were synthesized of which we may name $\alpha$-naphthalene-acetic acid, $\beta$-3-indole propionic acid and $\gamma$-3-indolebutyric acid. The above named acids were also found to be active in the *Avena* test (Avery, Burkholder and Creighton, 1937).

Hitchcock (1935) opened up a new field of investigation which has a close bearing on abnormal growth, by his experiments on the effect of introducing hetero-auxin and other synthetic growth-substances in lanoline or in water solution into such plants as tomato and tobacco. He found that various growth phenomena including epinastic response, cambial stimulation and adventitious root formation followed such application. Some synthetic compounds while producing the above effects in tomato plants were not active in the *Avena* test.

Certain members of the vitamin complex are being investigated in relation to abnormal and pathological growth and may prove of some importance in the interpretation of these phenomena. Thiamin (Vitamin $B_1$) which is produced by the normal plant is recognized as a root growth factor (Bonner, 1938).

## Techniques.

In quantitative work on growth-substance in plants, certain specialized techniques are used which may also be briefly outlined here to clarify statements as to methods used in investigations on abnormal growths produced by infective agents.

1. *Diffusion Technique and* Avena *Test.*

A modification of Went's original hormone diffusion method is used. The cut surfaces of normal (control) and abnormal tissues are placed on separate agar plates and the growth-substance present allowed to diffuse into the agar over a two-hour period. Each agar plate is then cut into twelve small blocks which are applied unilaterally to sets of decapitated *Avena* coleoptiles. Bromide prints record the negative curvatures after a two-hour period and the relative amounts of growth-substance diffusing from the normal and abnormal tissues can be assessed. This method has, however, been found to be of limited value for comparative investigations when dealing with green plants such as tomato.

2. *Ether Extraction of Growth Substance.*

A valuable method involving ether extraction developed by van Overbeek (1938), has been modified to suit conditions for investigations on abnormal growth. In the author's modification, normal and abnormal plant parts taken from "paired" plants are allowed to extract overnight at 4°C. in specially purified ether. The ether is evaporated on an electric hot-plate at a temperature below 60°C., down to 2 cc. in each container, transferred by pipette to centrifuge tubes which are standing in boiling water, so that the remainder of the ether is rapidly boiled off. Then 1 cc. of 1·5 per cent. agar is added to the tubes and thoroughly mixed with the residues. After two to three hours the agar is remelted and poured into small brass moulds. The chlorophyll-containing layer is sliced off and plates of standard size (8 x 10·7 x 1·5 mm.) cut from the agar. These again are cut into twelve blocks of equal size and applied unilaterally to *Avena* coleoptiles. Emphasis has been placed by some workers on the necessity for complete removal of auxins from plant tissues by repeated extractions but there appears no reason why the amounts obtained in a single extraction should not represent the relative amounts of free auxin present in the normal and abnormal tissues.

3. *The Pea Test.*

Briefly this consists of measuring the inward curvature of split portions of etiolated pea stems, which curvature occurs in the presence of certain growth-substances including indole-3-acetic acid. The method was developed by Went (1934). Thimann and Schneider (1939) have devised a similar but much more delicate test, using split coleoptiles of *Avena*.

4. *Treatments with Indole-3-acetic acid (Hetero-auxin) and other Synthetic Growth-Substances.*

These substances are frequently mixed with lanoline (method of Laibach, 1935) before application to stems or petioles of test plants. Concentrations ranging from 0·01 per cent. to 3·0 per cent. have been used. They may also be effectively introduced into test plants from small glass tubes drawn out to a capillary. The tube is filled with a water solution of the growth-substance and the capillary end of it inserted into the stem so that the liquid can slowly drain into the tissues (method of Zimmerman and Wilcoxon, 1935). Hetero-auxin can also be introduced into the plant through intact roots, by watering the soil with a strong water solution (Hitchcock and Zimmerman, 1935).

5. Phycomyces *Assay for Vitamin* $B_1$.

The fungus *Phycomyces Blakesleeanus* requires an external supply of thiamin (vitamin $B_1$) for growth and from this fact Schopfer and Jung (1937) have developed a quantitative biological assay. The fungal spores are sown into a basic thiamin-free medium to which thiamin extracted from test plant material is added. Comparison may then be made of the relative thiamin contents of normal and abnormal tissue on the basis of dry weight of fungus formed in a standard time.

## Overgrowth.

1. Crown Gall.

Some overgrowth conditions are due to strong parasitism, while certain others again arise from controlled parasitism (under which heading we can include symbiosis). One of the best known of the former and one on which a considerable volume of information is available, is Crown Gall. These galls arise on plants through parasitism by *Bacterium tumefaciens* Sm. & T. and this disease has been the subject of a great many studies, not only on account of its intrinsic interest but also because of its suggested analogy with cancerous conditions in man. Following infection, proliferation of cells leads to callus and gall formation, the galls being either primary or secondary. Other growth phenomena also occur and may be mentioned here, although their

physiology will be discussed later. Adventitious roots were frequently observed to be present in the vicinity of the growing galls. Smith, Brown and Townsend (1911), recorded their presence originally but detailed observations were first made by Brown (1929). She showed that Paris Daisy and Balsam plants in particular, reacted to inoculation by strong adventitious root formation in addition to gall formation. Epinasty of leaves of infected plants does not appear to have been recorded before 1937, although as Locke, Riker and Duggar (1937, 1938) point out, this phenomenon doubtless had frequently been observed by those studying Crown Gall.

Smith (1922), and Riker (1923) showed that the cambium was stimulated to activity in the presence of *B. tumefaciens*. Riker (1923), recorded the course of the early cell divisions initiating gall formation following infection. The walls were laid down in the portion of the mother cell near the intercellular spaces containing the bacteria. Up to the time when they could still be clearly observed, such cell walls formed a more or less distinct sheath round the bacteria.

There appears to be general agreement that the hyperplastic and hypertrophic growth, which, in various manifestations, generally follow Crown Gall inoculations, is due to some influence exerted by the bacteria. Crown Gall appears ideal as a test case for evolving an explanation of the physiological cause of the atypical cell multiplication, and prior to the development of the growth-substance theory, a considerable body of literature had already grown up concerning the possible importance of a great variety of chemical substances. These have been critically reviewed by Riker and Berge (1935), and will not be considered here, this review being concerned with the approach to Crown Gall through studies on growth-substance.

The view that growth-substance is concerned in Crown Gall formation arises from the fact that a close analogy exists between the stimulation effects induced in plants by such substances and the symptoms of the disease. Brown and Gardner (1936), and Kraus, Brown and Hamner (1936), demonstrated that indole-3-acetic acid could stimulate the multiplication of cells in French Bean plants, leading to the formation of galls which were structurally closely similar to those arising after infection by *B. tumefaciens*. Other points of similarity were recorded by Locke, Riker and Duggar (1938). Responses which suggest an increase in the amount of growth-substance present in infected plants include (1) epinasty of leaf petioles, (2) increased initiation of adventitious roots, (3) stimulated cambial activity, (4) inhibited development of certain buds, and (5) delayed abscission of senescent leaves.

With these similarities in mind, research on the mechanism of cell proliferation in Crown Gall has led to the formulation of the following hypotheses:—

(1) The parasite produces a growth-substance in the course of its metabolism which incites the cells of infected plants to abnormal division.

(2) The host plant reacts to the presence of the parasite by an excessive formation of auxins which induce the hyperplasia.

(3) Growth-substances produced by the parasite and by the host are jointly responsible for gall formation.

A viewpoint recently suggested, which has arisen out of negative evidence in relation to the above hypotheses, may also be listed here. It is to the effect that growth-substances of the auxin or hetero-auxin type are not important in relation to gall formation, but that other phyto-hormones, possibly of the vitamin complex, may be concerned.

It appears desirable to consider each of the above hypotheses in the light of the experimental evidence presented.

*Growth-substance Production by the Parasite.*

Nemec (1930) studied the effect of smearing some fresh culture of *B. tumefaciens* on the cut upper surface of chickory roots held under moist air conditions. He found that vigorous callus development was followed by adventitious root formation. Normally, buds developed from the cut upper surface but these were inhibited in the presence of the parasite. Nemec concluded that growth-substance from the bacteria was responsible for the effect. Brown and Gardner (1936) showed that it was possible to extract from the culture in which *B. tumefaciens* had grown, a growth-substance capable of inducing galls. These workers adopted the view that it was not the mere presence of the organism which leads to overgrowth, but rather the stimulus of certain products of its metabolism. In a later paper (Brown and Gardner, 1937), they suggested that secondary galls were also due to some stimulating substance which travelled through the stem, after being given off by the parasite at the site of the primary gall. Link, Wilcox and Link (1937) and Berthelot and Amoureux (1938) claimed that *B. tumefaciens* produced hetero-auxin in culture. The latter workers considered that this growth-substance played an important part in the genesis of Crown Galls. The former workers found that ether extracts of broth cultures of the parasite caused local killing in addition to stimulation effects, when applied to test plants. This indicated that some substances other than hetero-auxin were present in the crude ether extract. They concluded, however, that hetero-auxin was a chemical agent by means of which, possibly in conjunction with others, *B. tumefaciens* induced galls.

Gioelli (1940) tested the effect of filtered cultures of *B. tumefaciens* on plant tissue cultures of *Sterculia platanifolia*. He found that cambial stimulation occurred which was similar to that induced by hetero-auxin. This led him to conclude that the organism induced the proliferation by the action of hormones.

Locke, Riker and Duggar (1938, 1939*a*) first reported evidence which was in conflict with the hypothesis being considered. They demonstrated that both attenuated and virulent cultures of *B. tumefaciens* produced approximately the same amount of growth substance in media. There appeared to be no relation between the ability of the one and the inability of the other to form galls, and their ability to form growth-substance in culture. These workers concluded that it seemed better to reserve judgment regarding the importance of the role played by hetero-auxin in the development of Crown Gall. Other evidence adduced in support of their view was as follows:—(1) Indole-3-acetic acid was relatively ineffective in stimulating tissue inoculated with an attenuated strain of Crown Gall organism, whereas very small amounts of active substance diffusing from virulent gall inoculations were highly effective in this respect. (2) Responses of various plants, including bean and sunflower, to indole-3-acetic acid, did not parallel their response to Crown Gall bacteria. (3) The volume of culture which must be extracted and the number of bacteria present to give a small amount of growth-substance (optimum value 125 gammas per litre) is out of all proportion to the number of organisms present in a typical Crown Gall.

Grieve (1940) also reported that pathogenic and non-pathogenic cultures of *B. tumefaciens* produced approximately equal amounts of growth-substance. A similar result was obtained for *B. solanacearum, Aplanobacter michiganense and B. flaccumfaciens*. These results indicated that production of indole-3-acetic acid in media was not peculiar to bacteria which induced abnormal cell division, since *B. flaccumfaciens* did not produce any such effects in its host, the French bean. It is interesting that it is this plant which has proved so useful in demonstrating cell proliferation arising from infection by *B. tumefaciens*, by extracts of *B. tumefaciens* and by synthetic indole-3-acetic acid. The inference may be drawn from the above experiments that there is no necessary relation between growth-substance formation in media and in the host.

White (1942) claims that it is possible to produce a gall on a plant by implanting, at cambium level, a fragment of tissue derived from a bacteria-free tumour arising originally on a plant inoculated with *B. tumefaciens*. This would appear to provide further evidence that a product of bacterial metabolism is not causally involved in the atypical cell proliferation.

The writer is of the opinion that the balance of evidence indicates that it is unlikely that bacterially produced hetero-auxin plays any major role in atypical cell multiplication.

*Excessive Growth-Substance Production by the Host.*

Leonian (1937), in the course of a review of "Growth Hormones in Plants" (Boysen-Jensen), suggested in the case of Hairy root, tumours, galls and other pathological conditions, that such abnormal growths were the result of excessive production and concentration of auxins in an attempt to overcome the invader (sic), rather than to growth-substances furnished to the host plant by the pathogen. Link, Wilcox and Link (1937) also suggested that the parasite "probably not only furnishes a more or less continuous supply of hetero-auxones (hetero-auxin) but through abnormal growth or lethal effects of the host disturbs normal production, activation and transport of auto-auxones (plant auxins)". Link and Eggers (1941), using an ether extraction method in conjunction with the *Avena* test, presented evidence to show that there was a significantly greater amount of growth-substance present in gall tissue than in control tissue. In discussing this result they stated that "in addition to auxones, including auxins furnished by the parasite, the host cells—local and distant—also contribute to the hyperauxony of the affected organ".

Locke, Riker and Duggar (1938) showed that virulent cultures of *B. tumefaciens,* when inoculated into tomato stems above the inoculation points of an attenuated strain (which by itself only produced slight stem swelling) induced strong gall formation at the points of entry of the latter. These workers suggested therefore, that some substance from the tissue inoculated with the virulent strain, diffused down through the stem and stimulated cell division at the inoculation points of the attenuated strain. In similar experiments, hetero-auxin in lanoline paste applied in high concentration (30 mgm. per gram of paste), either failed to diffuse in quantity to the inoculation points of the attenuated culture or at least produced little effect there, as there was no significant increase in cell proliferation. A point of interest was that the stimulating effect of the virulent culture on gall development at the point of inoculation of the weak strain, was not exerted as markedly in the upward direction (Riker, 1940). This is perhaps only to be expected in accordance with the polar movement of growth-substance when in physiological concentration. The non-polar upward and downward movement of hetero-auxin in stems (see Brown and Gardner, 1936) apparently occurs when concentrations of this substance are high. The relative failure of hetero-auxin to induce increased cell proliferation, either at the point of inoculation of the attenuated strain or at any other point on the stem of tomato, is interesting in view of the results

of Brown and Gardner (1936) for French bean. The difference may be due, in part, to the host or to the mode of application. Locke, Riker and Duggar (1938, 1939*b*) agreed with Leonian (1937) that growth-substance was more likely to be a product of host cells under the influence of bacterial action rather than a direct bacterial metabolic product. They found that it was not possible, however, to distinguish between growth-substance from the foliage of the normal plant and from that in Crown Gall culture. The growth-substance extracted from the plant resembled indole-3-acetic acid in its sensitivity to acid and alkali. In this connection it may be noted that in recent papers Lefèvre (1938), and Haagen-Smit, Leech and Bergen (1941), have reported the presence of indole-3-acetic acid in higher plants.

*Gall Formation Due to Growth-substance Produced Both by Parasite and by the Host.*

Link, Wilcox and Link (1937) and Link and Eggers (1941), whose work has been reported in the preceding two sections, incline to the view that growth-substances are produced both by the parasite and by the plant. Each source contributes to the significantly greater amount of active substance which they found to be present in galled tissues.

*View that growth-substances of the Auxin and Hetero-auxin Type are not Important in Gall Formation.*

Riker, Birch Henry and Duggar (1941), using quantitative ether extraction methods, re-examined the question of growth substance content of inoculated and control tissue. In these experiments, involving a large number of *Avena* tests, they failed to find any significant difference between the auxin content of control and inoculated tissue. This held true over periods ranging from 1 to 16 days after inoculation. Similarly, no significant difference could be detected between the amounts of auxin diffusing from stems bearing galls and from control stems. A further interesting point was that when comparable plants were inoculated and grown at temperatures at which gall formation occurred (27°C.) and at which it did not occur (31°C.), there was found to be no significant difference in auxin production.

They therefore concluded that the gall formation was not due to the production of hetero-auxin or similar growth-substances as measured by the ether extraction and *Avena* technique. These negative results led them to examine the production of other active substances including vitamin $B_1$, biotin, flavin and pantothenic acid, in relation to the cell-stimulating property of the parasite. A preliminary note (Birch Henry, Riker and Duggar, 1942) indicates that vitamin $B_1$, as assayed by the *Phycomyces* method does not appear to have any causal role in Crown Gall development.

It should be pointed out that the experiments of Link and Eggers (1941), in which they showed that there was a significantly greater amount of growth-substance present in galled tissue, were on a comparable scale to those of Riker, Birch Henry and Duggar (1941). Since the results of these two groups of workers are diametrically opposed, it appears that the question as to whether there is present a greater amount of growth-substance in Crown Gall tissue, must be left open.

A consideration of the above lines of evidence indicates that the high hopes of the solution of the Crown Gall (cell proliferation) problem in terms of the production of hetero-auxin or similar growth-substance by the parasite, have not been realized. There are, however, very strong indications that some growth-substance mechanism is involved in infected plants. The most promising approach now, in the opinion of the writer, would be through a more thorough study of auxin and "food factor" relations in the normal and infected plant. The trend of modern research on the growth-substance theory, while maintaining the necessity of auxin for growth, indicates that even in the presence of adequate auxin, a second "food factor" is necessary. Schneider (1938) has shown this to be sugar in the case of *Avena*. It is possible, therefore, that the pathogenicity of the Crown Gall organism is related to its ability to supply some relatively simple "food factor" in the course of its metabolism. The local accumulation of this in addition to the usual "food factor" may upset the normal balanced growth at the point of entry of the parasite, leading to overgrowth. The work of Locke, Riker and Duggar (1938), in demonstrating that inoculation of tomato stems with a virulent strain, stimulated gall formation at the point of inoculation with an attenuated culture, may yet prove to be important in relation to such a "food factor" hypothesis.

### 2. Other Overgrowth Conditions Caused by Parasites.

Data on other overgrowth conditions caused by strongly parasitic organisms is at present meagre and in most cases has not advanced beyond the preliminary stage. Link, Wilcox and Link (1937) reported in the case of *Ustilago zeae*, causing Smut Galls on maize, and *Taphrina deformans* causing Peach Leaf Curl, that a substance giving tests for hetero-auxin could be extracted from the organisms. Moulton and Link (1940) in a short abstract, state that *Ustilago zeae* grown on a tryptophane-free medium is capable of producing "auxins". Link and Eggers (1941) refer to Moulton's work (Thesis 1941) on Smut Galls of Maize and state that, using delicate methods of extraction, more "auxin" was obtained from gall tissues than from healthy tissues. "Auxin" as used here appears to be used as a general term meaning growth substance.

## Overgrowth due to Controlled Parasitism (Symbiosis).

### Root Nodules of Leguminosae.

The root nodules of Leguminosae on account of their important relation to agricultural practice have attracted a great deal of attention from scientists. It is only recently, however, that attention has been focussed in greater degree on the mechanism of cell proliferation inducing the nodule formation. The condition is of interest to the pathologist because it lies on the border line of parasitism. It has long been debated whether the relationship of the nodule organism to its host plant constitutes an instance of true symbiosis or an instance of controlled parasitism. The work of Brenchley and Thornton (1925), in which they showed that when *Vicia faba* was grown on a boron deficient solution the bacteria attacked the host cells, followed by the researches of Thornton (1930*a*), in which he demonstrated that parasitism develops in the case of inoculated lucerne seedlings placed in the dark, swung opinion to the viewpoint of controlled parasitism. Under normal conditions, the organism and the plant live in equilibrium and the advantages are mutual.

Two of the fundamental problems involved are, firstly, how the bacteria enter the plant and, secondly, how the nodule is formed, that is, how the bacteria bring about the proliferation of cells. In order to understand how these occur it is first desirable to know something of the normal course of root infection. This has been clearly described and figured in the case of lucerne by Thornton (1936) and will be outlined here. The usual avenue of infection is via the root hairs and the earliest stage detected by Thornton was the formation of a small colony of the bacteria close to the distal end of the root hair, the tip of which usually becomes coiled into a short spiral. Infection takes place only in this deformed region, this deformation being apparently a necessary prelude, and manifests itself by the formation of a thread of bacterial zoogloea passing down the interior of the root hair. Nodules become visible to the naked eye within twenty-four hours after infection of the root hair. Nodules of clover and lucerne consist of a mass of proliferating cells mostly in the cortex but penetrating also into the pericycle (Thornton, 1930*b*). The central cells swell and become infected with bacteria and by the time that the nodule is a week old the cytoplasm of the infected cells in the central region, becomes filled with bacteria. Thornton and Rudorf (1936) state that there is present at the distal end of a healthy legume nodule, a cap of meristem cells by whose continued division the nodule grows in length. This meristem is also concerned with the differentiation of the lateral endodermis and vascular strands.

Sufficient has now been given of morphology and structure to allow us to consider possible mechanisms of the formation of the nodule. It was discovered as early as 1900 by Hiltner that filtered secretions of the bacteria could induce deformation of the root hairs indicating that the organism excreted some active substance into its medium. Molliard (1912) working with rhizobia from bean, recorded the fact that sterile bacterial secretions could cause abnormal growth effects in roots of *Pisum*. He found that roots of peas allowed to grow in such solutions were retarded in growth, showed increased cell division, radial enlargement in the pericycle and deformation of cortical cells as compared with control roots which had grown in non-inoculated media. Molliard concluded that the organism excreted some active substance into its media which brought about conditions similar to those whoch obtained in actual infection.

Secretions from *Rhizobium meliloti* were shown by Thornton (1936) to stimulate both the production and growth in length, and deformation of the root hairs of lucerne. The substance was non-specific in its action, in that filtrates of cells from one cross inoculation group, deformed root-hairs of plants belonging to another group (McCoy, 1932). Its action in increasing production and length of root hairs would bring it into the class of growth-substances. As Wilson (1940) points out, the deformation of the hair on the basis of hormone in the filtrate is not so easily explained. One would not expect differential rates of growth as the concentration of the hormone should be the same on all sides of the root hairs. An interesting point arises here in that this substance stimulates growth in length of root hairs; all growth-substances so far tested have an inhibiting effect on elongation in root tissue. The possibility must be considered that root hairs are organized in a similar fashion to and consequently react in a similar way to the cells of coleoptiles on the application of growth substance. There appears, however, to have been no investigations on the effect of auxins on root hairs.

Thimann (1936, 1939) was the first to examine the question of the mechanism of nodule formation in the light of the auxin theory of growth. He postulated the following series of events after the bacteria enter the root tissues:—Small amounts of an auxin, among other substances, are produced in the course of bacterial metabolism, especially when the organisms attack carbohydrate or protein in the invaded cells. This substance causes enlargement of the cells in which it is produced and also being readily diffusible enters the pericycle behind the cortical tissue inhabited by the bacteria and there stimulates growth and division giving rise to the first stages of a lateral root initial. In the presence of continued auxin production, however, this potential lateral root is prevented from elongating; instead its cells increase in size isodiametrically while certain of the

uninfected cells are stimulated to division by auxin diffusing out of the infected area. In this way he considers "a shapeless mass of parenchymatous tissue is produced which is essentially a lateral root prevented from elongating." The evidence on which Thimann based his hypothesis was as follows:—(*a*) The auxin activity of young nodules (2 to 3 mm. in diameter) was of the order of 10 to 12 plant units per nodule for three hours' diffusion. (*b*) Auxin was demonstrated to be present not only in the apical meristematic region but also in the basal portion which consists of infected cells. (*c*) Application of auxin to very young lateral roots resulted in complete inhibition in growth elongation, swelling due to radial elongation of cortical cells, together with divisions in the cambium and pericycle. (*d*) Indole-3-acetic acid was produced in culture media containing tryptophane in which rhizobia had grown. Thimann's general conclusions were that the course of nodule formation involves the production of indole-3-acetic acid by the bacteria which probably liberate it in the course of breakdown of tryptophane present in the nodules. He cites the work of Molliard (1912), noted earlier, pointing out that the effect of indole-3-acetic acid on the root is exactly the same as that of the sterile filtrate tested by Molliard.

Wilson (1937, 1940) discussing Thimann's work indicated that the idea of indole-3-acetic acid being the long sought stimulant was attractive, but rightly pointed out that more experiments were required. He also considered that further evidence was necessary to determine whether secretions from the root nodule bacteria would cause a response similar to that obtained when synthetic hetero-auxin was applied. Regarding this last point it would appear that the work of Molliard (1912) demonstrates, in the case of *Pisum* roots, effects of filtrates which are similar to those induced by auxins. However, if Thimann's hypothesis is to apply generally, it is still necessary to demonstrate nodule-forming phenomena, other than root curling, when bacterial secretions are applied in the case of lucerne, clover, etc. A further difficulty which may be mentioned is that many investigators believe that the structure of the nodule does not indicate a modified lateral root as Thimann states. Fred, Baldwin and McCoy (1932) discuss the evidence for and against this conception of its nature, and conclude:—"It (the root nodule) is distinctly not a modified lateral root, for it has no central cylinder, root-cap nor epidermis. Furthermore, it does not digest its way out from the cortex of the main root but remains covered with a considerable layer of cortical parenchyma."

It has been shown by a number of workers, Link (1937), Chen (1938), Thimann (1939), and Georgi and Beguin (1939) that various species of *Rhizobium* produced indole-3-acetic acid in culture. The position has been complicated, however, by the fact that both effective and ineffective strains of nodule bacteria

and also organisms which live as contaminants in the nodules, all produce comparable amounts of growth-substance. Thus Chen (1938) found that effective and ineffective strains of clover bacteria when growing vigorously in liquid culture produced similar amounts of growth-substance as assayed by Went's split pea test (Went, 1934). Old laboratory strains that had lost the ability to infect and produce nodules were, however, found to produce less. Georgi and Beguin (1939) reported that four species of *Rhizobium* in culture produced indole-3-acetic acid. According to them the ineffective strains appeared to be more efficient growth-substance producers than the effective strains. *B. radiobacter,* a contaminant living in the nodules, was also found to produce growth-substance. These workers consequently question whether indole-3-acetic acid plays a causal role in nodule growth.

Chen, Nicol and Thornton (1940) appear to accept the view that initiation and maintenance of the apical meristematic cap of the nodule is due to growth-substances produced by the bacteria. Discussing the difference between nodule production by effective and ineffective strains, they state that the arrested growth in the ineffective nodules is due to the stopping of cell division in the apical cap, which in turn would appear to be related to the early arrest or decrease in growth of the contained bacteria. The decrease in bacterial growth might be taken to indicate that less growth-substance would be produced and consequently less cell division would occur. These workers made the interesting observation that juice from roots (of peas and soy beans) bearing effective nodules gave better growth for the organism in culture than did control juice, and that juice from roots of plants with ineffective nodules gave growth significantly poorer than control juice. Chen, Nicol and Thornton (1940) suggest that the stimulating effect was possibly concerned with the products of nitrogen fixation. It seems possible, however, that it may be some active growth-substance which is produced in the plant as a reaction to infection by effective strains and that as it can stimulate bacterial growth it may also be concerned in the cell proliferation.

Link and Eggers (1940), and Link, Eggers and Moulton (1941) present evidence to show that more growth-substance (as assayed by the *Avena* test) can be obtained by ether extraction from nodules of kidney bean and garden pea, and to a lesser extent for soy bean, than from denodulated roots, while less still could be obtained from roots grown in sterilized quartz sand. They conclude from this that nodules of bean, soy bean and pea have greater and different auxin contents than the roots which bear them and that these in turn have greater auxin content than the roots when grown in sterilized substrates. This represents a more cautious view than that expressed earlier by Link

(1937) when he stated that indole-3-acetic acid was one of the chemical agents, if not the primary one, responsible for the formation of nodules in certain leguminous plants. Thimann and Schneider (1939) although not comparing nodules with denodulated roots, pointed out that nodules of *Phascolus vulgaris* were very active in producing growth-substance as tested by the quartered coleoptile test.

In conclusion we may say, that in the case of root nodules of Leguminosae, there appears to be no doubt that the bacteria secrete some chemical substance which causes the increase in length and deformation of root hairs in nature and also under laboratory conditions. According to Nicol (1938) the chemistry of this substance is still obscure. Its physiological activity, however, would appear to put it in the class of growth-substances. The mechanism inciting cell proliferation in the nodule is still in doubt. The hypothesis that indole-3-acetic acid (produced by bacterial metabolism in the plant) is causally related to cell proliferation is not favoured by the balance of evidence. Nor does it appear likely that the substance, referred to above, which is responsible for root hair deformation, can be the cause of the overgrowth.

There appears to be no conflict of evidence so far on the question of heightened growth-substance content in the nodules, and it therefore appears likely that a hormone mechanism is involved. This increased growth-substance would appear to be developed by the plant as a response to the bacterial infection.

Further research on the identity of the stimulating substance for "effective" nodule bacteria found in the roots of pea and soy-bean, will, it is believed, prove helpful in the solution of the problem.

## Adventitious Root Formation.

Adventitious roots develop as a host reaction in tomato and certain other plants following infection by *Bacterium solanacearum*, *Bacterium tumefaciens*, *Aplanobacter michiganense* and *Fusarium bulbigenum* var. *lycopersici*. Such disease-induced roots, which generally show on the stem surface as small nodular projections, are to be distinguished from those which may develop on healthy plants under glasshouse conditions.

Hunger (1901), Smith (1914, 1920), Bryan (1915), and Grieve (1936, 1940) have reported on their occurrence in the case of *B. solanacearum*. In this disease the adventitious roots on tomato plants develop characteristically over several internodes along the path of the primary bundles, spreading later to the secondary tissues. Plants which show the reaction include tomato, African Marigold, garden Nasturtium and Sunflower (Grieve, 1940).

The presence of induced adventitious roots in plants such as Balsam, tomato and Paris Daisy infected with *B. tumefaciens* has been recorded by Brown (1929), Link, Wilcox and Link (1937) and Locke, Riker and Duggar (1938). Grieve (1940) pointed out that tomato plants artificially inoculated with *B. tumefaciens* show fewer adventitious roots, which are also more localized in distribution, than is the case for *B. solanacearum*. These differences appear to be related to the different distribution of the bacteria in the two diseases. Again, adventitious root formation in tomato, arising from infection with *A. michiganense*, ranges from abundant to scanty. Smith (1914, 1920) first reported their occurrence but found them to be relatively few in number. He concluded that this was due to the fact that this phloem parasite rapidly invaded the root primordia.

Fisher (1935) first mentioned root initials as being associated with infection by *Fusarium lycopersici* and this observation was confirmed by Wellman (1941).

That a stimulus of some sort was involved in adventitious root formation in the case of *B. solanacearum* was envisaged by Hutchinson (1913) and Smith (1920). Grieve (1936) pointed out the similarity of the reaction to that induced by ethylyene and carbon monoxide gases (Crocker *et al* 1932, Zimmerman *et al* 1933), and indole-3-acetic acid (Hitchcock, 1935a). Locke, Riker and Duggar (1938) noted this also for *B. tumefaciens* infections and Wellman (1941) for Fusarium Wilt.

The extended development of the adventitious roots along the vascular bundles in the case of tomato and African Marigold plants infected by *B. solanacearum*, made these plants suitable hosts for an examination of the mechanism of adventitious root formation. The disease differs from Crown Gall in that it is possible to ascertain the relation of the bacteria to the developing roots. Thus Grieve (1940) pointed out the following relations of *B. solanacearum* to the induced roots:—

(*a*) Adventitious roots frequently commenced to develop ahead of advancing columns of bacteria in vessels.

(*b*) Development of the root primordia, once initiated, continues to the stage where the root becomes visible as a nodule at the surface of the stem, even though the bacteria during this period gradually block all the vessels nearest the incipient root.

(*c*) Where vessels are rapidly filled with bacteria, no adventitious roots develop.

This histological study also established the fact that the action of the bacteria, in the case of tomato at least, was not always excited from a distance as Hutchinson (1913) and Smith (1920)

believed. Nevertheless it strongly suggested that the bacteria were inducing the roots either directly by the production of some stimulatory substance or indirectly by their interference with the metabolism of the plant.

Grieve (1936, 1939, 1940) reported the extraction of a physiologically active substance from culture media containing glucose, peptone and mineral salts in which the bacterium was grown. This extract in crude form gave positive tests for hetero-auxin and induced adventitious roots on application to tomato and African Marigold.

Experiments with virulent and non-virulent cultures of *B. solanacearum* showed, however, that they produced approximately equal amounts of growth-substance. The same held true for pathogenic and non-pathogenic cultures of *A. michiganense*, *B. tumefaciens* and *B. flaccumfaciens*.

The suggestion that *B. solanacearum* produced growth-substance by acting on naturally occurring or artificially introduced tryptophane in the xylem was experimentally tested but no evidence of such production was obtained. This led the writer to doubt whether the bacteria initiated the adventitious root primordia through the medium of hetero-auxin production, and led to the formulation of the view that the mechanical blocking by the bacteria might induce the effect through disturbance of the normal hormone movement.

Experiments involving cutting and blocking of the xylem with inactive substances gave results which supported this view. Ether extractions of growth-substance which were assayed by the *Avena* test, showed, however, no significant difference in the amounts present in comparable healthy and infected plant parts.

Further experiments are required to check this, but the writer is of the opinion that significant auxin differences are not necessary, since the amount of auxin necessary to induce adventitious root formation *in vivo* is minute. One must also envisage the using up of some of this auxin in bringing about the root formation.

The problem of adventitious root formation in the case of *B. tumefaciens* is bound up with the general problem of gall formation and the conclusions reached there apply. The evidence of Locke, Riker and Duggar (1938, 1939), of Riker, Berch, Henry and Duggar (1941) and of Grieve (1940) makes it appear reasonably certain that hetero-auxin formation by the parasite is not causally related to adventitious root formation. Opinion also is still divided on the question of relative amounts of auxin in galled tissues as compared with comparable healthy plant parts.

With regard to *A. michiganense* no investigation appears so far to have been made, apart from the observation that pathogenic and non-pathogenic cultures of this organism produce approximately the same amounts of growth-substance (Grieve, 1940).

Wellman's paper on Fusarium Wilt (1941) merely reported adventitious root formation. The course of the disease here is not unlike that of Bacterial Wilt (*B. solanacearum*) in so far as symptoms are concerned and should prove a suitable subject for research on the mechanism of adventitious root formation.

It would be interesting to know whether the toxin produced by *Fusarium bulbigenum* var. *lycopersici,* has any bearing on the reaction.

## Growth Movements—Epinasty and Hyponasty of Leaves.

Epinasty of leaves seems to be generally associated with adventitious root formation, both occurring as symptoms in the same disease, e.g., plants infected with *B. solanacearum* (Hunger 1901, Smith, 1920), *B. tumefaciens* (Locke, Riker and Duggar, 1938), and *Fusarium bulbigenum* var *lycopersici* (Wellman 1941). Leaflet epinasty has also been recorded as a primary symptom in the case of Rose Wilt Virus (Grieve, 1941) and there is a strong tendency toward it in young tomato plants infected with tomato Spotted Wilt. Hyponasty of leaves has been observed in plants infected by *Bac. phytophthorus.*

The only investigations so far available on leaf epinasty induced by parasites are those by the author (Grieve, 1936, 1939, 1940, 1943).

The reaction in the case of infection by *B. solanacearum* was shown to be an irreversible growth reaction and invasion of one lateral trace sufficed to induce it.

An experimental investigation of possible causes of the response showed that toxin production, ammonia production and hydrogen-ion effects were not involved. Experiments on mechanical blocking gave largely negative results (1939), although in later experiments (1940) there appeared to be some evidence which favoured it.

A growth-substance which gave the tests for hetero-auxin, was found to be produced in media in which the bacterium had grown, and this substance induced epinasty in leaves of young tomato plants.

No significant difference between the growth-substance content of comparable portions of infected and control stem parts of tomato plants showing leaf epinasty could be detected by the

ether extraction method. Examination of the hormone distribution in the upper and lower halves of reflexing petioles has, however, shown a significantly greater amount in the upper halves. In normal petioles there is a greater concentration in the lower half (Grieve, 1943).

In 1939 the writer made the suggestion that the normal petiolar position was conditioned by a balanced hormone mechanism and that the disturbance of this would lead to epinastic response. The results given above show that the growth reaction of epinasty is associated with an increased concentration of auxin towards the upper surface. This redistribution of growth substance, which has also been demonstrated to occur in the case of leaf epinasty associated with Crown Gall, is induced by the bacteria. No definite conclusion as to how the redistribution of growth substance in the basal part of the petiole is effected, can yet be made. As the writer pointed out (1939), however, the balance of the normal growth controlling mechanism at the base of the petiole is very delicate. This is reflected by the fact that ethylene in one part in 10 million of air, as well as very small amounts of growth substance from bacterial cultures, suffice to disturb it. It is not unlikely, therefore, that even a small stimulus from the invading organisms can initiate a chain of reactions leading to the redistribution of hormone with consequent epinastic response.

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